Model of Ca^{2+} concentration controlled by sarcoplasmic reticulum of skeletal muscle, using the state transition

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Abstract- **[Ca^{2+}] in a muscle cell is controlled by the sarcoplasmic reticulum (SR) that releases Ca^{2+} through the channels, takes up Ca^{2+} by the pumps on the SR membrane, and stores up Ca^{2+} with Ca^{2+} binding protein called calsequestrin (CS).**

This report proposed a model that represents [Ca^{2+}] in a muscle cell controlled by the SR using a state transition probability model in which one state means that protein in the SR is binding ligands, and the other is releasing them. The proposed model consists of 4 modules: calsequestrin, voltage dependent Ca^{2+} release channels, Ca^{2+} induced Ca^{2+} release channels, and Ca^{2+} pumps.

Estimating the amount of Ca^{2+} both released and pumped up with the model, it was indicated that [Ca^{2+}] rapidly increases from the static state as soon as nerve impulses arrive at a muscle. We further reveal that the fact that Ca^{2+} pumps are located apart from Ca^{2+} release channels has an important influence on generating a Ca^{2+} spike signal.

Keywords – **E-C coupling, Ca^{2+} release channel, Ca^{2+} pump, calsequestrin, sarcoplasmic reticulum**

I. Introduction

A nerve impulse arriving at a muscle, Ca^{2+} concentration ([Ca^{2+}]) goes up in the muscle cell, which causes actin filaments to slide on myosin filaments; thus muscle force is developed. The amount of developed force depends greatly on [Ca^{2+}] in the cytosolution, which is controlled by Ca^{2+} release channels and Ca^{2+} pumps on the sarcoplasmic reticulum [1][2].

The cytosolic [Ca^{2+}] control is achieved mainly by 4 processes. (1) One is a process of storing Ca^{2+} by calsequestrin (CS), because having a lot of Ca^{2+} binding sites, many Ca^{2+} are storable in the sarcoplasmic reticulum (SR). (2) The second process is that in which transverse tubule (TT) on the muscle fiber receives newly-arrived nerve impulses by its voltage sensors, and transmits the information to specific type of Ca^{2+} channels (V-channel) on the SR membrane, along with TT conformation change and increasing local [Ca^{2+}]. (3) The third process is that in which the increasing local [Ca^{2+}] induces Ca^{2+} release channels (C-channel) to open their gates. Then Ca^{2+} in the SR is released out of the SR in the cytosolution. (4) The last process is that in which Ca^{2+} pumps on the longitudinal SR membrane capture the released Ca^{2+} into the SR. Thus the cytosolic [Ca^{2+}] decreases again and settles in a static state.

Although several models of [Ca^{2+}] control in muscle cell have been proposed up to now, most of these are described from chemical reaction equation point of view using binding constant k or K_D [3][4]. Since some single molecular measurement techniques have been developed, which enables stochastic behaviors on single molecular level to be measurable, a new type of model constructed from a level of single molecular stochastic behaviors can be utilized for understanding micro phenomena such as temperature dependency.

From this point of view we construct a Ca^{2+} control model using state transition probabilities, in which one state means that protein in the SR is associating with ligands, and the other state means disassociating. The proposed model consists of 4 modules corresponding to the 4 processes of [Ca^{2+}] control. Using the model we calculate time course of cytosolic [Ca^{2+}] that is obtained from both Ca^{2+} efflux amount through the SR membrane and afflux amount into the SR.

II Model of a ligand binding site on a protein

We first consider a simple case in which there exists only a ligand and a site on protein, and the site is associating or disassociating with the ligand stochastically. Let the duration times that the site is associating with the ligand and disassociating with it, be stochastic variables in accordance with exponential distributions with the expected value σ and τ, respectively. We will call τ the mean lifetime. In a case there exist more than one of ligands, the number of which depends on the concentration [L], whereas the site can not

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Abstract

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associate with more than one ligand simultaneously. The probability λ transiting from a state in which the site disassociates with a ligand to the other state in which the site associates with a ligand during unit time Δt, and the probability of μ transiting to the reverse direction are represented as follows:

$$\left.\begin{aligned}\lambda &= 1-(1-\sigma^{-1}\Delta t)^{[\mathrm{L}]}\\ \mu &= \tau^{-1}\Delta t\end{aligned}\right\} \qquad (1)$$

Diagram of such a state transition is illustrated in Fig.1. We here define the binding affinity as $A_{ff}=\sigma/\tau$.

III. Model Structure

Let us focus on a half sarcomere. We approximate the form as a cylinder with a height of 1.1 μm, a radius 0.5 μm and a volume of 0.86 μm^3 [5]. The half sarcomere is divided into 4 parts: CS, V-channels, C-channels and Ca^{2+} pumps as illustrated in Fig.2. The ratios of cytosol and SR to a half sarcomere in volume are supposed to be 0.77 [6] and 0.091[5], respectively.

A. Calsequestrin

A calsequestrin molecule has 31 Ca^{2+} binding sites [7], and the density of binding sites is 31mM in the SR [8]. The ratio of the number of calsequestrin binding Ca^{2+} to the total number of Ca^{2+} binding sites on calsequestrin for various $[Ca^{2+}]$ is measured by experiment [7]. Parameters σ_{CS}^{-1} and τ_{CS}^{-1} for calsequestrin included in our model are estimated by a nonlinear optimization method so that the ratio calculated by the model can correspond to the experimental data. The estimated parameters σ_{CS}^{-1}, τ_{CS}^{-1} depend on the initial values, which are randomly set for the nonlinear optimization, whereas the binding affinity $A_{ff}=\sigma_{CS}/\tau_{CS}$ of calsequestrin to Ca^{2+} calculated from the estimated σ_{CS}^{-1} and τ_{CS}^{-1} have almost equal values with no dependency to the initial values, which suggests the affinity is an essential parameter with no redundancy. Following simulations are done using estimated values $\sigma_{CS}^{-1}=0.828\times10^{-2}$, $\tau_{CS}^{-1}=0.239\times10^{-3}$ per 10^{-2} msec.

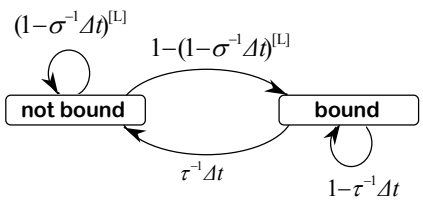

Fig.1 State transition of a ligand binding site

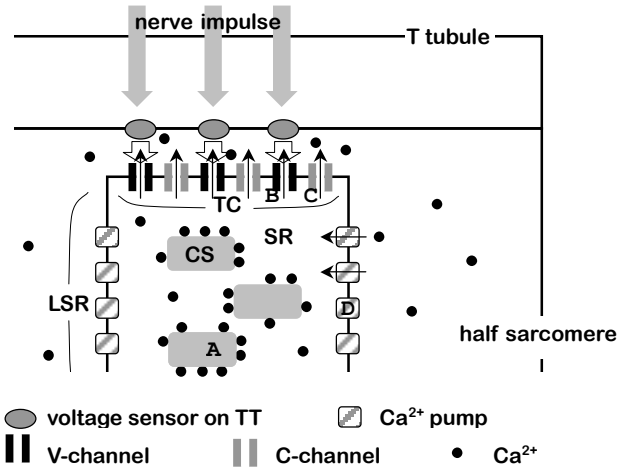

Fig.2 Model construction

B. V-channel

Ca^{2+} channels are located on terminal cisternae (TC), which is a part of SR membrane apposed to the TT. The radius of a Ca^{2+} channel is about 15nm [9]. Since the TC occupies 0.049 of a sarcomere in volume, there are about 600 Ca^{2+} channels in a half sarcomere. Ca^{2+} channels can be classified into 2 types as the functions: V-channel and C-channel. The V-channels and C-channels are located almost alternately [2].

Depolarizing of TT is transmitted to V-channels on the SR along with some mechanical conformation changes. The detail of this mechanism has not been elucidated yet. When the signal arrives at the V-channel, the channel gate opens, and then Ca^{2+} flows from the SR to sytosol. The Ca^{2+} current through the channels depends on $[Ca^{2+}]$ at both cytosol and the inner SR. The membrane voltage potential E_m is assumed to be described as a Nernst equation, and thus Ca^{2+} current $I_{channel}$ is postulated to compute as shown in Eq.(2).

$$I_{channel}=k_I G E_m=k_I G\frac{RT}{zF}\ln\frac{[\mathrm{Ca}^{2+}]_{cyt}}{[\mathrm{Ca}^{2+}]_{SR}}, \qquad (2)$$

in which, G, R, T, z, F, $[Ca^{2+}]_{cyt}$, and $[Ca^{2+}]_{SR}$ are channel conductance, gas constant, absolute temperature, electric charge of ion, Faraday constant, $[Ca^{2+}]$ in cytosol and $[Ca^{2+}]$ of the inner SR, respectively. We assumed that channel conductance G is 313pS [11], and $I_{channel}$ through the

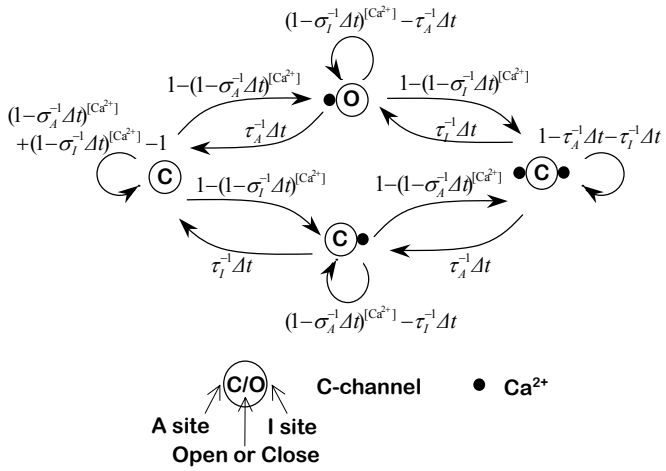

Fig.3 State transition of a C-channel

V-channel is calculated with a coefficient k_I.

We here introduce some hypotheses: the signal arrival time is a Gaussian stochastic variable, V-channel open immediately when signal arrives, and the open lifetime is a stochastic variable with exponential distribution.

C. C-channel

Ca^{2+} through V-channel elevates cytosolic $[Ca^{2+}]$. The open probability of C-channels depends on cytosolic $[Ca^{2+}]$. It has been considered that a C-channel has two Ca^{2+} binding sites, one of which is an activate site (A-site) and the other is an inactivate site (I-site). A C-channel keeps opening while A-site is associating with Ca^{2+} and I-site is disassociating with Ca^{2+}. The state transition diagram of a C-channel is expressed in Fig. 3.

The lifetime τ_A during A-site is associating with Ca^{2+} is estimated as 382 msec using Fig.5 appeared in [11]. The transition parameter σ_I^{-1} in respect to I-site, is calculated as 0.00459 per 10^{-2} msec using experimental data in Fig.3 appeared in [11]. The other transition probabilities σ_A^{-1} and τ_I^{-1} are estimated, by calculating open state probability using experimental data [12]. So σ_A^{-1} and τ_I^{-1} are estimated as 0.0905 and 0.0167 per 10^{-2} msec, respectively.

D. Ca^{2+} pump

There are a lot of Ca^{2+} pumps on the longitudinal SR membrane. Since the ratio of the longitudinal SR to a sarcomere is 0.06 in volume [5], there are 40,400 Ca^{2+} pumps in a half sarcomere. After a Ca^{2+} pump binds two Ca^{2+} in cytosol, these Ca^{2+} are transported to the inner SR side on the membrane, and then released immediately. When a Ca^{2+} pump binds only one Ca^{2+}, the binding affinity of the other site becomes very high. For convenience sake in this model it is postulated that as soon as the first Ca^{2+} binding is caused the second site binds Ca^{2+} immediately, and released these Ca^{2+} are transported to inner SR side. Thus the state transition of a Ca^{2+} pump can be represented as in Fig.1.

Ca^{2+} transition parameters σ_{pump}^{-1}, τ_{pump}^{-1} of a Ca^{2+} pump are estimated as 0.353 and 0.430×10^{-2} per 10^{-2} msec, respectively, using experimental data [13].

Because Ca^{2+} release channels are placed apart from the pumps, released Ca^{2+} arrives at the pumps with a time delay. In this model, the delay time is supposed to be 1.1 msec in maximum, considering Ca^{2+} diffusion constant 7×10^{-4} mm^2sec^{-1}[14]

IV. Model Simulation and Discussions

It is assumed that a delay time from when an activated signal arrives to when V-channel open is a Gaussian stochastic variable with the mean 0.5 msec and the variance 0.5 $msec^2$. The lifetime of open V-channels distributes exponentially with the mean 0.5 msec. The number of open V-channels is shown in Fig.4.

The following simulations are executed with the time step 10^{-2} msec. The initial cytosolic $[Ca^{2+}]$ is set to be 10^{-7} M which is its static concentration.

Using the model the time course of cytosolic $[Ca^{2+}]$ is estimated as shown in Fig.5, which is considered as the shape of Ca^{2+} spike. However after 14 msec $[Ca^{2+}]$ has a tendency to slightly increase. The presence of proteins binding Ca^{2+} in the SR such as parvalbumin and troponin, are not considered in our model. Thus it is likely that the proteins depress cytosolic

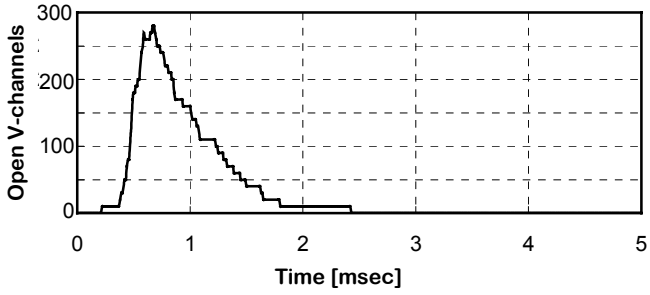

Fig.4 The number of openV-channels

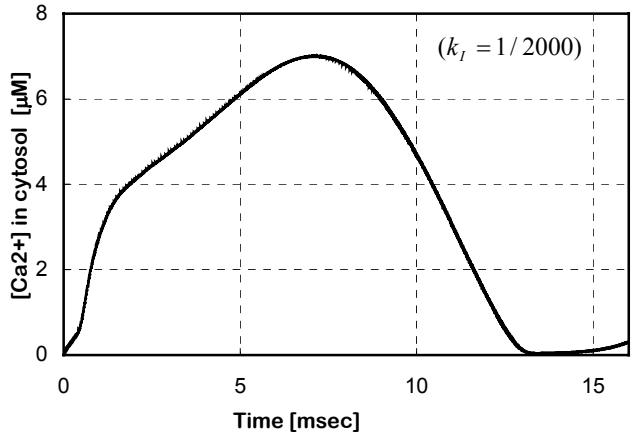

Fig.5 Estimated [Ca^{2+}] in cytosol

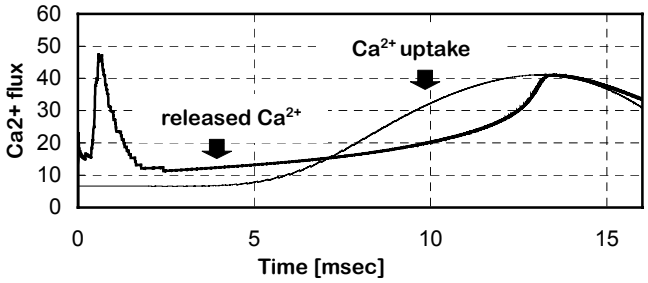

Fig.6 The flux of released Ca^{2+} from V- and C-channels, and taken Ca^{2+} into SR

[Ca^{2+}] lower than the estimated [Ca^{2+}] in Fig.5.

The released Ca^{2+} flax from both V- and C-channels during 10^{-2} msec is shown with a thick line in Fig.6. The thin line in Fig.6 represents the afflux of uptake Ca^{2+} by Ca^{2+} pumps. It can be seen that the C-channels release Ca^{2+} a few msec later than V-channels. Furthermore, Ca^{2+} pump uptake arises earlier than C-channels release Ca^{2+}. It seems that the cytosolic [Ca^{2+}] is controlled by the fine balance between the Ca^{2+} afflux by Ca^{2+} pumps and efflux from the channels. It is likely that keeping the fine balance and generating the Ca^{2+} spike is owing to the moderate distance between Ca^{2+} release channel and Ca^{2+} pumps.

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